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AMRL-TR-69-3

AD691474

200607072



**ELECTROENCEPHALOGRAPHIC AND
BEHAVIORAL STUDIES OF
MONOMETHYLHYDRAZINE TOXICITY
IN THE CAT**

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JUNE 1969

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences—National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89-544, "Laboratory Animal Welfare Act," August 24, 1967.

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Foreword

This research was performed under Contract AF 33(615)-2822, by the Department of Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, California 90024. The work was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry," Work Unit 018, "Research on the Subconvulsive Effect of Air Force Compounds on the Nervous System," from January 1967 to November 1968, for the Toxicology Branch, Toxic Hazards Division, Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, Ohio.

The experiments were conducted jointly by M. B. Sterman, PhD, Chief, Neuropsychology Research, Veterans Administration Hospital, Sepulveda, California, and Assistant Professor, Departments of Anatomy and Physiology, UCLA, R. W. LoPresti, PhD, Post-doctoral Fellow, Brain Research Institute, UCLA, and M. D. Fairchild, PhD, Research Pharmacologist, Veterans Administration Hospital Long Beach, California, and Assistant Professor, Department of Pharmacology, UCLA. Kenneth C. Back PhD, was contract monitor for the Aerospace Medical Research Laboratory.

This technical report has been reviewed and is approved.

C. H. KRATOCHVIL, Colonel, USAF, MC
Commander
Aerospace Medical Research Laboratory

Abstract

The toxicity of monomethylhydrazine (MMH) administered intraperitoneally in the cat was studied by reference to behavioral and neurophysiological indices. The acute toxicity LD_{50} value for MMH was established as 15 mg/kg, and the CD_{50} as 7 mg/kg. Doses of 18, 9 and 5 mg/kg were then studied systematically in an effort to classify lethal, convulsive and subconvulsive symptoms. For these doses, a preconvulsive syndrome was described involving recurrent and sustained symptoms including vomiting, panting, rapid respiration, viscous salivation, hyperactivity and subcortical seizure activity. The onset latency of these symptoms was directly related to dose. Several lines of evidence suggested at least a partial independence between the biochemical and neurophysiological events responsible, on the one hand, for convulsions, and on the other for this preconvulsive syndrome. Convulsions were specifically delayed or prevented in animals trained to suppress movement through the use of a special EEG conditioning technique.

Section I

INTRODUCTION

Valuable information concerning the central nervous system and behavioral effects of the toxic propellant 1, 1-dimethylhydrazine (UDMH) has been gained from a battery of neurophysiological and performance tests developed in our laboratory. Current studies represent the initial stages of a similar evaluation of monomethylhydrazine (MMH). The performance studies have been reported in a separate communication (Serman, Fairchild, and VanTwyver, 1968). Evaluation of changes in cerebral neuroelectric responses is scheduled for the near future.

The objectives of the present experiments were twofold. Our primary goal was to determine the acute, convulsive and subconvulsive toxicity values of MMH administered intraperitoneally in the cat. The acute toxicity LD₅₀ value for intravenous administration in mice and rats is approximately 33 mg/kg and, for dogs, 12 mg/kg (MLCR No. 52). We were interested in this and more information with regard to the cat. Specifically, we wished to determine the range of characteristic behavioral and electroencephalographic effects of MMH toxicity and to classify their order of occurrence as a function of dose.

Our secondary objective was to test an hypothesis derived from our previous studies of UDMH. It was proposed that learned inhibition of motor behavior could significantly delay or prevent the occurrence of central nervous system (CNS) seizure activity in cats exposed to convulsive doses of MMH.

Neurophysiological studies of UDMH have shown that convulsive doses result in a graded increase of the excitability of cortical neurons responding to afferent sensory bombardment (Goff et al., 1967). This process was attributed to a progressive failure of the inhibitory interneurons which normally modulate the excitability of axodendritic synapses. In this regard, UDMH appears to act like strychnine by blocking the release of inhibitory transmitter substances from cortical interneurons (Eccles, 1963). Studies of seizure thresholds in limbic and other subcortical structures indicated a similar increase in excitability at this level (Fairchild and Serman, 1964). The picture which emerged with UDMH was a gradual increase in sensory excitability coupled with a gradual decrease in motor inhibition. It was proposed that a sensorimotor positive feedback condition thereby resulted and led inevitably, at doses of 20 mg/kg and above, to central seizures and convulsions.

The findings suggested that an enhancement of the central process of motor inhibition might effectively delay the occurrence of seizures by interruption of this situation. Studies of paralyzed cat preparations showed that the absence of proprioceptive sensory feedback significantly delayed the occurrence of electrocortical seizure activity (Goff et al., 1967). Paralysis, however, is an impractical therapy for this condition. An approach to the problem in normal animals would be of greater value. Assuming that a similar process occurs with MMH exposure, this drug was administered to normal behaving cats that had previously been trained to suppress movement.

Section III

RESULTS

The characteristic sequence of events attendant upon the intraperitoneal administration of MMH in the cat consisted initially of an abrupt period of restlessness followed shortly by vomiting. This was followed, in turn, by a succession of definitive symptoms with both recurrent and continuous manifestations. The recurrent symptoms included persistent vocalization, motor hyperactivity, and subcortical seizure discharge. Vomiting and defecation were seen often during these periods. During the intervals between these recurrent episodes, the animals were extremely alert, but inactive. They appeared distressed and unwilling to move. The continuous manifestations were EEG desynchronization, panting, rapid respiration, and viscous salivation. At 9 and 18 mg/kg the characteristic sequence was terminated by wild escape behavior leading directly into convulsions. These symptoms of MMH toxicity were dependent, in degree and in latency of onset, upon the dose administered (Table I). Table I shows that the impact of the drug, at all three dose levels, was registered within the first 30 minutes following injection.

TABLE I
CHARACTERISTIC SYMPTOMS OF MMH TOXICITY:
RELATIONSHIP OF DOSE TO ONSET LATENCY

(Time in Minutes)

Symptoms	5 mg/kg (N=3)	9 mg/kg (N=3)	18 mg/kg (N=3)
Restlessness	28.0± 3.6	10.0±11.3	5.3±0.6
Vomiting	30.3± 2.1	22.0± 5.0	7.0±2.6
Vocalization	38.3± 7.2	30.5± 1.5	21.0±3.0
Panting	52.5± 4.5	35.7± 2.1	24.3±5.0
Salivation	62.5± 4.5	43.5±13.5	25.5±8.5
Hyperactivity	67.3±15.0	40.3±10.3	24.3±3.0
Subcortical Seizure	145.0±77.5	*	—
Escape Behavior	—	54.0±13.5	28.0±1.7
Convulsions	—	54.7±13.1	29.0±1.7

*Unclear

5 mg/kg: In addition to the symptoms described in Table I, the most striking feature of this subconvulsive dose was its effect upon the electrical activity of the brain. Following the occurrence of vomiting, and for a continuous period of 4-7 hours thereafter, the EEG showed a sustained pattern of low-voltage fast (desynchronized) activity characteristic of extreme attention and alertness (Figure I). Although the animal showed frequent alternation between active and quiet behavior, there was no evidence of relaxation or sleep. Reference to data from control animals (Sternman et al., 1965) indicated that such extended periods of desynchronized EEG activity and the lack of relaxation or sleep were abnormal.

Section II

METHODS

Pilot studies of MMH administered intraperitoneally in the cat established the CD_{50} at 7 mg/kg, and the LD_{50} at approximately 15 mg/kg. Therefore, to determine lethal, convulsive and subconvulsive symptoms, animals were studied systematically with doses of 18, 9 and 5 mg/kg.

Eight adult cats, weighing 2.5-5 kg each, were surgically prepared for chronic electrophysiological and behavioral study. Under deep barbiturate (Nembutal) anesthesia, the animals were placed in a stereotaxic instrument, and small burr holes were drilled in the skull over sensory and motor cortex and at appropriate locations for insertion of deep recording electrodes. Small stainless steel screws ($\frac{1}{16}$ in. diameter) were threaded into the skull for cortical recording, and pairs of 10 mil. insulated stainless steel wires, separated by 1 mm, were lowered stereotaxically into a variety of subcortical nuclei. Particular emphasis was placed on thalamic sensory and motor structures and on pathways and way-stations in pyramidal and extrapyramidal motor systems. Leads from these electrodes were connected to a 20-contact plug and the entire assembly fixed to the cat's skull with a crown of dental cement. Wound margins were sutured, and the animals were allowed to recover with careful postoperative care.

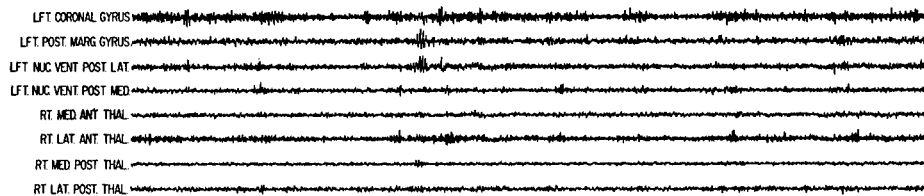
After recovery, 8-hr recordings were obtained in each animal from both cortical and deep electrodes with simultaneous observation of behavior. Animals were then administered 5 (subconvulsive), 9 (convulsive) and/or 18 (lethal) mg/kg doses of MMH by intraperitoneal injection. Some animals received all three doses in ascending order and others received only one or two doses. Six animals received the 9 mg/kg dose. Three of these animals were from the group prepared for these studies, and three were drawn from a different experiment. This experiment was concerned with the neurophysiology of behavioral inhibition.

Our laboratory has described a specific EEG rhythm which can be recorded over the sensorimotor cortex of the cat whenever spontaneous or trained suppression of movement occurs (Roth, Sterman and Clemente, 1967). This "sensorimotor rhythm," or SMR, appears to be a direct indicator of the operation of a central process of motor inhibition. The most profound evidence for this is derived from experiments in which this EEG rhythm is specifically reinforced by milk in hungry animals. A conditioned EEG response develops in association, behaviorally, with a total suppression of movement (Sterman and Wyrwicka, 1967; Wyrwicka and Sterman, 1968).

Thus, the three animals drawn from these experiments had, several months earlier, received extensive SMA conditioning experience. This training has been found to profoundly influence EEG and motor patterns over long periods of time (Sterman, Wyrwicka, and Clemente, 1968).

CAT 13

A. PRE DRUG: QUIET



B. POST DRUG (5mg/kg MMH): QUIET

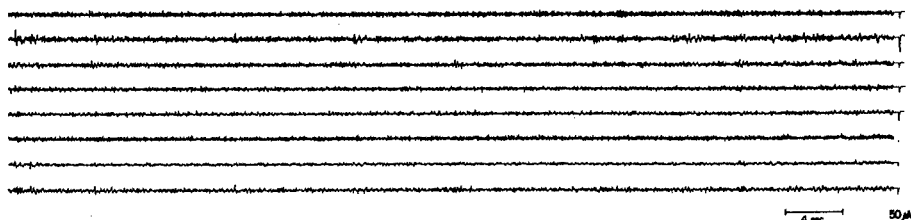


Figure 1. Typical segments of cortical and subcortical electrical activity recordings in a quiet animal before and 1 hour after the intraperitoneal injection of 5 mg/kg MMH. Note the dramatic absence of any slow-wave activity in the latter.

At this dose there was no evidence of seizure activity in the cerebral cortex. However, subcortical leads in several nuclei of the thalamus showed periodic local seizure discharge (Figure 2). These were accompanied often by strange motionless postures, with the animal staring off into the distance or gazing intently at his own lifted paw. Occasionally, they followed a brief period of hyperactive behavior. After a period of 7 hours, both EEG and behavior patterns appeared to be normal once again.

CAT 13: 5mg/kg MMH

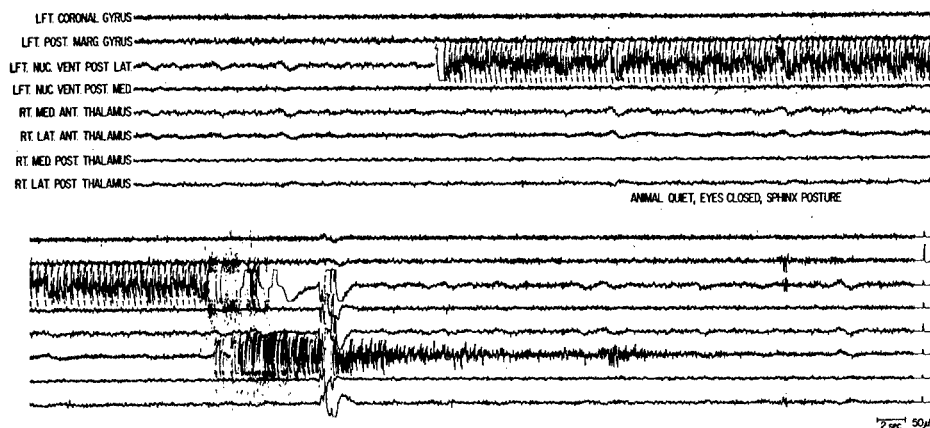


Figure 2. Spontaneous subcortical seizure discharge in a quiescent animal after administration of 5 mg/kg MMH. In this instance the seizure discharge is seen to shift from the left ventrolateral thalamus to the right anterolateral thalamus. During the latter phase the animal was seen to raise his forelimb slowly and remain immobile for several seconds. Such subcortical seizure patterns were not always distinguishable because of the movement artifacts caused by accompanying motor activity.

9 mg/kg: The pattern of symptoms associated with this dose of MMH was identical to that described above for 5 mg/kg; however, the latency of onset and magnitude were altered (Table I). The events which constitute the characteristic symptoms of toxicity were significantly intensified and appeared with approximately two-thirds the delay seen at 5 mg/kg. They included, additionally, escape behavior and convulsions. With 9 mg/kg the animals displayed, in addition, a moderate ataxia together with, in later stages, a persistent generalized motor tremor. Barbiturate anesthesia was typically administered after convulsions, but the postictal period was studied in several animals without this treatment. Recurrent convulsions and protracted symptoms of toxicity persisted for up to 24 hours.

18 mg/kg: With the exception of vomiting, which occurred within the first 10 minutes following injections at this dose, acute toxic symptoms appeared abruptly and continuously after approximately 20 minutes. Ataxia was a consistent feature of the pattern seen at this dose. These symptoms were intense and sustained until convulsions, which occurred with remarkable consistency after 27-31 minutes. The course of events was so rapid that several of the characteristic symptoms appeared only briefly, or not at all, in advance of the seizures. In animals not treated with barbiturate, convulsions occurred repeatedly within intervals of approximately 5 minutes. In some instances seizure discharge was initiated in subcortical sites and propagated rapidly throughout the brain (Figure 3). Five or six toxic-clonic convulsions occurred in rapid succession and were followed by death within one hour after injection.

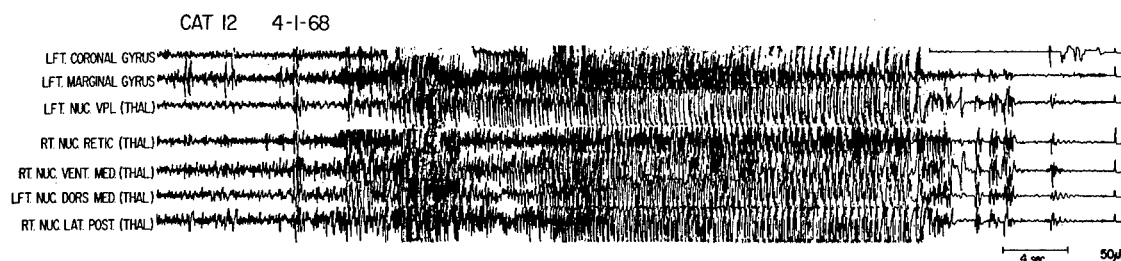


Figure 3. Generalized CNS seizure discharge associated with convulsions following the administration of 18 mg/kg MMH. Although diffuse spiking activity was seen preceding overt convulsions, the onset of generalized seizures was occasionally initiated at subcortical sites. Note post-ictal depression following seizure discharge.

A comparison between untrained cats and animals with SMR training at doses of 9 mg/kg showed a dramatic difference in the onset latency of convulsions. Whereas the latency of toxic symptoms, such as vomiting, panting, etc., did not differ reliably between these groups, escape behavior and convulsions were delayed substantially (Table II). EEG recordings from pretrained animals showed a dramatic enhancement of the SMR when compared to records obtained from untrained animals (Figure 4). This enhanced SMR activity was associated with the delay in development of general CNS seizures. It is apparent, therefore, that learned facilitation of motor inhibition through techniques directly influencing its central representation in the brain can effectively delay, or perhaps prevent, the incapacitating consequences of exposure to convulsive doses of MMH.

TABLE II
COMPARISON OF ONSET LATENCY OF MMH
TOXICITY SYMPTOMS WITH 9 MG/KG IN
UNTRAINED CATS AND CATS GIVEN MOTOR
INHIBITORY TRAINING
(Time in Minutes)

<i>Symptoms</i>	<i>Mean + S.D.</i>	<i>Mean + S.D.</i>
	<i>Untrained (N=3)</i>	<i>SMR Trained (N=3)</i>
Restlessness	10.0±11.3	17.0±12.5
Vomiting	22.0 ±5.0	19.3±15.2
Vocalization	30.5± 1.5	33.3± 8.5
Panting	35.7± 2.1	39.0±11.8
Salivation	43.5±13.5	33.5± 4.5
Hyperactivity	40.3±10.3	*
Subcortical Seizures	*	*
Escape Behavior	54.0±13.5	164.5±74.1
Convulsions	54.7±13.1	167.6±74.1
	(range=40-67)	(range=80-220)

*Unclear

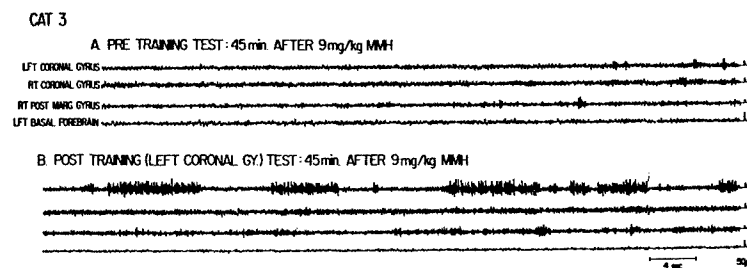


Figure 4. Comparison of sensorimotor rhythm activity from left coronal gyrus following administration of MMH in a cat before and after conditioning of this rhythm. In the pretraining test this animal showed convulsions 57 minutes after injection, whereas the post-training test yielded convulsions 220 minutes after injection.

Section IV

DISCUSSION

These studies have established the acute toxicity LD_{50} value for intraperitoneal administration of MMH in cats as 15 mg/kg. The CD_{50} value was 7 mg/kg. Characteristic symptoms of toxicity at lethal, convulsive and subconvulsive doses included vomiting, vocalization, panting and rapid respiration, viscous salivation, and motor hyperactivity. At the subconvulsive dose tested here, subcortical seizures were an additional feature appearing usually 1-2 hours after administration. With convulsive and lethal doses, escape behavior and generalized CNS seizures terminated this characteristic sequence of symptoms. The onset latency of these symptoms was found to be directly related to dose in a near linear fashion. Moreover, the latency values obtained were remarkably stable, particularly at the convulsive and lethal dose levels.

Subconvulsive exposure to MMH induced the entire sequence of symptoms short of convulsions themselves, and did so within the first hour following administration. In this respect, MMH differed in kind, as well as degree, from UDMH. With UDMH a similar sequence of symptoms was recorded in the cat (Fairchild and Stermann, 1964). With convulsive exposures their onset latency was similarly related to dose, although these latencies were more than twice those for MMH. However, unlike MMH, subconvulsive doses of UDMH produced none of the preconvulsive symptoms, and deleterious effects could only be disclosed with sensitive behavioral testing. These related toxic compounds probably initiate the same chain of biochemical and neurophysiological events which lead to convulsions. However, preconvulsive symptoms can occur without eventual convulsions at 5 mg/kg doses of MMH. Moreover, several animals tested with 18 mg/kg MMH showed only vomiting, hyperactivity and seizures without vocalization, panting, or salivation. These facts suggest that some of the preconvulsive symptoms may be at least partially independent of the convulsions themselves.

From the standpoint of protection, perhaps the most interesting finding of these studies was the significant delay of convulsions resulting from learned suppression of movement. It may be recalled that the animals used in this phase of the experiment had not received SMR training for over three months prior to the drug tests. In recent pilot studies we have specifically trained animals for several weeks following tests of convulsive doses of MMH and then retested immediately afterwards. These animals showed no spontaneous convulsions even though the preconvulsive syndrome occurred intact. Therefore, this training apparently provided an effective protection against the most devastating consequences of this compound. Note, however, that this training involved a more-or-less direct intervention into the central nervous system since it was dependent upon reinforcement of a central electrical event and not merely motor behavior. We are presently seeking similar EEG phenomena in man.

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DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) University of California, Los Angeles Department of Anatomy and the Brain Research Institute Los Angeles, California 90024		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	
		2b. GROUP N/A	
3. REPORT TITLE ELECTROENCEPHALOGRAPHIC AND BEHAVIORAL STUDIES OF MONOMETHYLHYDRAZINE TOXICITY IN THE CAT			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates) Final Report, January 1967-November 1968			
5. AUTHOR(S) (First name, middle initial, last name) M.B. Sterman, PhD R.W. LoPresti, PhD M.D. Fairchild, PhD			
6. REPORT DATE June 1969		7a. TOTAL NO. OF PAGES 8	7b. NO. OF REFS 10
8a. CONTRACT OR GRANT NO. AF 33(615)-2822		9a. ORIGINATOR'S REPORT NUMBER(S)	
b. PROJECT NO. 6302			
c. Task No. 630202			
d. Work Unit No. 630202018		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) AMRL-TR-69-3	
10. DISTRIBUTION STATEMENT This document has been approved for public release and sale; its distribution is unlimited.			
11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY Aerospace Medical Research Laboratory Aerospace Medical Div., Air Force Systems Command, Wright-Patterson AFB, OH 45433	
13. ABSTRACT The toxicity of monomethylhydrazine (MMH) administered intraperitoneally in the cat was studied by reference to behavioral and neurophysiological indices. The acute toxicity LD50 value for MMH was established as 15 mg/kg, and the CD50 as 7 mg/kg. Doses of 18, 9, and 5 mg/kg were then studied systematically in an effort to classify lethal, convulsive and subconvulsive symptoms. For these doses, a preconvulsive syndrome was described involving recurrent and sustained symptoms including vomiting, panting, rapid respiration, viscous salivation, hyperactivity and subcortical seizure activity. The onset latency of these symptoms was directly related to dose. Several lines of evidence suggested at least a partial independence between the biochemical and neurophysiological events responsible, on the one hand, for convulsions, and on the other for this preconvulsive syndrome. Convulsions were specifically delayed or prevented in animals trained to suppress movement through the use of a special EEG conditioning technique.			

14.	KEY WORDS	LINK A		LINK B		LINK C	
		ROLE	WT	ROLE	WT	ROLE	WT
	Pharmacology Toxicology Monomethylhydrazine (MMH) Behavioral indices Neurophysiological indices EEG conditioning Cats						